PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BW352R/RCGE	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/IB2005/050714	International filing date *(day/month/year)* 28.02.2005	Priority date *(day/month/year)* 27.02.2004
International Patent Classification (IPC) or national classification and IPC C07K14/415, C12N15/29, C12N15/62, A61K39/36		
Applicant CONSIGLIO NAZIONALE DELLE RICERCHE et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.12.2005	02.02.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hillenbrand, G Telephone No. +49 89 2399-8428

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2005/050714

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-17 as originally filed

Sequence listings part of the description, Pages

1-7 as originally filed

Claims, Pages

1-22 received on 07.12.2005 with letter of 06.12.2005

Drawings, Sheets

1-9 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-22
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

D1: WO 02/20790 A (CONSIGLIO NAZIONALE DELLE RICERCHE; GERACI, DOMENICO; COLOMBO, PAOLO;) 14 March 2002 (2002-03-14)

D2: COSTA M A ET AL: "CDNA CLONING, EXPRESSION AND PRIMARY STRUCTURE OF PAR J I, A MAJOR ALLERGEN OF PARIETARIA JUDAICA POLLEN" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 341, no. 2/3, 21 March 1994 (1994-03-21), pages 182-186, XP002049655 ISSN: 0014-5793

D3: DURO G ET AL: "cDNA cloning, sequence analysis and allergological characterization of Par i 2.0101, a new major alergen of the Parietaria judaica pollen" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 399, 1996, pages 295-298, XP002191113 ISSN: 0014-5793

D4: COLOMBO P ET AL: "THE ALLERGENS OF PARIETARIA" INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, vol. 130, no. 3, March 2003 (2003-03), pages 173-179, XP009036928 ISSN: 1018-2438

D5: BONURA A ET AL: "HYPOALLERGENIC VARIANTS OF THE PARIETARIA JUDAICA MAJOR ALLERGEN PAR J 1: A MEMBER OF THE NON-SPECIFIC LIPID TRANSFER PROTEIN PLANT FAMILY" INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, vol. 126, no. 1, September 2001 (2001-09), pages 32-40, XP001037388 ISSN: 1018-2438

D6: MENNA T ET AL: "CHARACTERIZATION OF A DODECAPEPTIDE CONTAINING A DOMINANT EPITOPE OF PAR J 1 AND PAR 0 1, THE MAJOR ALLERGENS OF P. JUDAICA AND P. OFFICINALIS POLLEN" ALLERGY, MUNSKGAARD, COPENHAGEN, DK, vol. 54, no. 10, 1999, pages 1048-1057, XP009038929 ISSN: 0105-4538

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Novelty (Article 33.2 PCT) and inventive step (Article 33.3 PCT)

Having regard to the documents cited in the International Search Report the subject-matter of claims 1-22 appears to be novel (Article 33.2 PCT).

Documents **D1** and **D5** disclose already Parietaria judaica NS-LTP antigen variants (Parj1) modified by substitution of cysteine residues with Ser at position 4, 29 and 30 (see in **D1** SEQ ID NO: 8 and 10 and in **D5** Fig. 1). The DNA and amino acid sequence of "wild-type" Parj1 and Parj2 were known to the skilled person since 1994 and 1996, respectively (see **D2 and D3).** The major allergens of Parietaria have been disclosed in **D4**. Finally, fusion proteins containing a dominant (allergenic) epitope of Parj1 were prepared in **D6.**

In view of the detailed and convincing arguments of the experienced representative of the applicant, Mr. Claudio Germinario, in paragraphs 4.5-4.9 of his reply dated 06.12.2006 - in combination with the figures of the present application which show a surprising/unexpected effect of the claimed matter over the cited prior art, inventive activity involved with the claimed matter can be acknowledged (Article 33(3) PCT). In view of the objections raised hereinafter this authority, however, proposes to include the subject-matter of claim 3 into claim 1 when entering the European Regional Phase.

Re Item VIII

Certain observations on the international application

The subject-matter of claims 1-2 is to broadly and imprecisely worded and thus does not comply with the requirements of Article 6 PCT (support). The applicant claims fusion proteins comprising different allergens belonging to the non-specific Lipid Transfer Protein (ns-LTPs) family. This family of proteins is characterized by their ability to transport lipids through membranes in vitro and these proteins appear to be present in all vegetal organisms. The applicant claims fusion proteins comprising an unlimited number of possible allergens derived from this extremely broad family of proteins (see claims 1-2). On the other hand, the claimed surprising/advantageous effect was only obtained with fusion proteins comprising allergens derived from regions of the known Parj proteins. In view of the large number of allergens falling under the broad definition used by the applicant in claims 1-2, this authority came to the conclusion that with respect to claims 1-2 there is lack of support for such extremely broadly worded claims and, in addition, considers that with respect to the subject-matter of claims 1-2 there is lack of sufficiency of disclosure.

CLAIMS

1. A fusion protein characterised in that it comprises the amino acid sequences of different allergens belonging to the non-specific Lipid Transfer Protein (ns-LTPs) family, in that said sequences lack one or more of the four disulphide bridges present in the sequence of the wild type allergens, at least one in the amino terminal region comprised between the amino acid residues 1 and 30 and in that said sequences maintain essentially the same length of the sequences of wild type allergens.

2. The fusion protein according to claim 1, characterised in that the amino acid sequence of each of the allergens is independently mutated by elimination or substitution of one or more cysteine residues involved in the formation of a disulphide bridge.

3. The fusion protein according to any one of the claims 1 to 2, characterised in that it comprises the allergens Parj1 and Parj2 of the *Parietaria judaica* species.

4. The fusion protein according to any one of the claims 1 to 3, characterised in that the amino acid sequence of each of the allergens is independently mutated by elimination or substitution of one or more cysteine residues in positions corresponding to the positions 4, 14, 29, 30, 50, 52, 75 and 91 of the amino acid sequence of Parj1 and/or Parj2 allergen.

5. The fusion protein according to any one of the claims 1 to 4, characterised in that it contains the amino acid sequences of the Parj1 and Parj2 allergens, both independently modified by substitution of cysteine residues with Asn, Ser, Thr, Ile, Met, Gly, Ala, Val, Gln or Leu residues in positions 29 and 30 or 4, 29 and 30 or 29, 30, 50, 52.

6. The fusion protein according to claim 5, having the amino acid sequence SEQ ID NO: 4.

7. A nucleotide sequence comprising the DNA coding for the fusion protein according to any one of the claims

1 to 6.

8. The nucleotide sequence according to claim 7 comprising the nucleotide sequence SEQ ID NO: 3.

9. An expression or cloning system comprising the nucleotide sequence according to claims 7 or 8 flanked by suitable sequences for controlling, promoting and regulating the expression.

10. A host cell transformed by means of the expression or cloning system according to claim 9.

11. The fusion protein according to any one of the claims 1 to 6, for use in a diagnostic or therapeutic treatment method *in vivo* and/or *in vitro*.

12. The fusion protein according to claim 11, for use as hypoallergenic immunologic agent in the specific immunotherapy (SIT) treatment of allergies.

13. The fusion protein according to claim 11, for use in the treatment of rhinitis, conjunctivitis, urticaria, angioedema, eczema, dermatitides, asthma, anaphylactic shock.

14. The fusion protein according to claim 11, for the preparation of DNA vaccines.

15. A pharmaceutical composition comprising the fusion protein according to any one of the claims 1 to 6 and a pharmaceutically acceptable excipient.

16. The pharmaceutical composition according to claim 15 in the form of solution, suspension, emulsion, cream, ointment or implant.

17. The pharmaceutical composition according to claim 15, for a parenteral, subcutaneous, intramuscular, intravenous, topical, oral administration or for subcutaneous implantation.

18. A method of preparation of the fusion protein according to any one of the claims 1 to 6, characterised in that suitably mutated amino acid sequences of different allergens are produced and linked directly or via a spacer for chemical synthesis or by expression, in the form of fusion protein, in genetically modified host

cells.

19. The method of preparation according to claim 18, characterised in that host cells are transformed with an expression vector comprising the DNA coding for the amino acid sequences in fused form, mutated via site-specific mutagenesis in codons coding for cysteine residues.

20. The method of preparation according to claim 19, characterised in that one or more cysteine residues are substituted with Asn, Ser, Thr, Ile, Met, Gly, Ala, Val, Gln or Leu residues.

21. The method of preparation according to any one of the claims 18 to 20, characterised in that one or more cysteine residues in position 29, 30 or 4, 29, 30 or 29, 30, 50, 52 are substituted with alanine or serine residues.

22. The method of preparation of a pharmaceutical composition according to any one of the claims 15 to 17, characterised in that the heterodimer protein is mixed in an immunologically active amount to a pharmaceutically acceptable excipient.